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Effect of Cis-Trans Isomers and Related Physical Properties of Monounsaturated Lipids on Shortening Power

Aneta Joyce Ostrander
University of Tennessee, Knoxville

To the Graduate Council:

I am submitting herewith a dissertation written by Aneta Joyce Ostrander entitled "Effect of Cis-Trans Isomers and Related Physical Properties of Monounsaturated Lipids on Shortening Power." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Food Science and Technology.

Ada Marie Campbell, Major Professor

We have read this dissertation and recommend its acceptance:

John T. Smith, Grayce E. Goertz, Bernadine Meyer

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

July 15, 1968

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Accepted for the Council:

Helton P. Smith
Vice President for
Graduate Studies and Research

EFFECT OF CIS-TRANS ISOMERS AND RELATED PHYSICAL PROPERTIES OF MONOUNSATURATED LIPIDS ON SHORTENING POWER

A Dissertation

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

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by

Aneta Joyce Ostrander

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ABSTRACT

The effect of cis-trans isomers on the shortening power of lipids was investigated. Lipid samples studied included: a commercial vegetable oil (control), oleic acid, triolein, elaidinized oleic acid, elaidinized triolein, two samples in which elaidinized oleic acid was substituted at different levels for oleic acid, and two samples in which elaidinized triolein was substituted at different levels for triolein. Breaking strengths of plain pastry wafers were used for estimating the shortening power. Melting point, surface tension, interfacial tension and viscosity measurements were made on the lipid samples. Lipid composition analyses included gas-liquid and thin-layer chromatography.

Except at the 100 percent level of substitution, wafers containing fatty acids had lower breaking strengths than those containing triglycerides. Breaking strengths of wafers containing samples substituted at the 100 percent level of elaidinized material were greater than those of wafers containing samples substituted at lower levels. Melting points of the lipids were positively correlated with breaking strength and with percent trans isomers. Surface tension and viscosity of fatty acid samples were lower than those of triglyceride samples. Values for interfacial tension did not differ consistently. The moisture levels of the doughs made from fatty acids were higher than those made from triglycerides. Doughs containing elaidinized

lipids at the 100 percent level of substitution were higher in moisture content than the other doughs. Relationships among various measurements are discussed. A multiple regression equation is presented for predicting breaking strength values.

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I. INTRODUCTION

In the last few decades concern about the type of fat consumed in the diet has resulted in a change in manufacture of shortening. In the past, shortenings were obtained mainly from animal sources. Today shortenings generally are made from vegetable oils. Hydrogenation is used to convert the oils to a semisolid state. During hydrogenation numerous positional and geometric isomers are formed (1, 2, 3, 4, 5, 6). By the beginning of the 1960s considerable attention had been directed toward the relationship of dietary fat composition and atherosclerosis (7). At this time changes in the processing of hydrogenated shortenings were occurring. Many manufacturers began producing shortenings that had a higher level of unsaturation than previous shortenings (8). In general these shortenings have a high iodine value similar to that of olive oil but are semisolid at room temperature (5).

In 1966 the consumption of shortening made from vegetable oils in the United States was about 1899 million pounds (9). Assuming the concentration of trans fatty acids in shortenings to range from 20 to 30 percent (4, 5), Americans would consume annually about 340 to 510 million pounds or 1.7 to 2.6 pounds per capita of trans fatty acids from shortenings. These shortenings may be consumed in a variety of food products such as pastry.

Information as to the functional properties of elaidinized fat in food products was not found in a search of the available literature.

It is known that elaidinization of fats and oils results in a higher melting point without loss of unsaturation, producing a semisolid fat from an oil. This type of isomerization gives a fat that is more resistant to oxidation than the original oil.

Many of the functional roles of fat have been studied. Of particular interest is its role as a shortening agent. The ability of a fat to produce a tender product has been called shortening power. Several theories have been presented to explain the shortening power of fats. One characteristic studied has been the degree of unsaturation (10, 11). The more unsaturated fats, being liquid at room temperature, seem to have a greater covering power, which has been related to shortening ability. Plasticity of the fat also has been related to shortening power (3). Fats containing triglycerides with a wide range of melting points appear to have a greater plasticity and shortening power than fats containing triglycerides with a narrow range of melting points. The effect of raising the melting point of oils, without changing unsaturation, on the functional role of shortenings has not been reported. It would seem that with the increased consumption of trans fatty acids the relationship between geometric configuration at the double bond and the shortening power of fats in baked foods should be studied. The effect of cis-trans isomers and related physical properties of monounsaturated lipids on shortening power is reported here. Shortening power was measured by breaking strength of pastry.

II. REVIEW OF LITERATURE

Several theories have been presented to explain the shortening power of fats. In the last three decades little work has been reported in this area. During this time a change has occurred in the manufacture of shortening. Shortenings generally are made from oils and in the process of hydrogenation cis-trans isomerization occurs. Elaidinization and shortening power of fats are reviewed.

Elaidinization

Elaidinization of fats and oils has been studied for more than a hundred years. In 1819 it was observed that olive oil could be converted to the consistency of pork fat by such a process (12). Straub and Malotaux (13) also found that it was possible to convert oils to semi-solid margarines by elaidinization. A variety of catalysts was studied (14). Of these, selenium was found by Bertram (15) to be useful in a practical method to change oils to semisolid margarines. These elaidinized fats were found to have greater resistance to oxidation than either ordinary or hydrogenated oils and fats (16). Stronger emulsification power has been observed with sodium elaidate than with sodium oleate (17).

Today a renewed interest in elaidinization has been reflected in the increased number of articles appearing in the technical literature. As the national interest in the use of unsaturated oils and margarines increases, so do problems of providing stability and plasticity.

Elaidinized fats and oils have better keeping quality and higher melting points than oils, without the loss of unsaturation that is characteristic of hydrogenated shortening. With possible commercial application of elaidinization to natural fats and oils, the question of the influence of the trans isomers on the functional role of shortening in food products is raised.

Although considerable information has been obtained on the formation of trans isomers during hydrogenation (12, 14), less attention has been directed to elaidinization solely as a means of changing the characteristics of fats. Earlier reports (13, 16) on the effect of elaidinization on fats and vegetable oils and their fatty acids should be accepted with some reservation because of limitations of the analytical methods used.

Much of the work on elaidinization has consisted of attempts to explain the mechanism in relation to the catalyst used. Elaidinization agents studied have included oxides of nitrogen (18, 19, 20), selenium (15, 18, 21), sulfur dioxide (18, 20, 22), nickel (6, 20) and mercaptans (23). Litchfield and co-workers (19) stated that oxides of nitrogen and selenium are the most widely used agents.

In studies of the use of oxides of nitrogen, the presence of undesirable nitrogenous by-products has been observed. Litchfield et al. (19) studied cis-trans isomerization during the use of nitrous acid and reported a method for removal of nitrogenous by-products.

Blekkingh and co-workers (18), using selenium or nitrogen trioxide, found no migration of the double bond during elaidinization. Hydrogenation of mono-ethenoid acids, however, resulted in the formation of both positional and geometric isomers (2, 6). The higher of two levels of nickel catalyst used resulted in increased amounts of trans isomers (6).

Several studies indicated the occurrence of cis-trans isomerization during catalytic hydrogenation (2, 3). Mabrouk and Brown (5) assayed six margarines and five shortenings which represented samples typically manufactured in the United States. With one exception all the margarines and shortenings tested contained trans isomers. The amounts of trans isomers ranged from 22.7 to 41.7 percent. In a more recent study, Jones et al. (4) isolated 27 percent trans isomers in a hydrogenated soybean oil and only 19 percent in a hydrogenated winterized oil.

Recently the use of infrared analysis (4, 19, 24), thin-layer chromatography (25, 26) and gas-liquid chromatography (27) has made composition analyses more reliable. Although elaidinized margarines (13) and hard butters containing elaidic acid (28) are made, information as to their effect on food products was not found in a search of the available literature.

Shortening Power

Functional role of neutral lipids in foods. The function of fats and oils in flour mixtures is to enhance flavor and richness or to modify texture and tenderness. The colloquial term "shortening" expresses the major result of the addition of fat, because it produces a product that breaks off abruptly in "short" rather than longer shreds.

The effect of the addition of larger amounts of fat is best seen in various forms of pastry in which the mechanical separation of the flour particles prevents the formation of a continuous phase of hydrated protein. If pastry were made without shortening, the addition of water to the flour would result in the development of gluten which upon manipulation would form an elastic dough becoming hard and tough on baking.

Factors related to shortening power. The use of fats and oils in pastry crust has been known for several centuries (29). By the early 1900s little if any work had been published regarding the physical and/or chemical characteristics that are related to the shortening power of a fat.

In 1923 Platt and Fleming (30) reported that when the study of shortening power was initiated in their laboratory in 1914 nothing could be found in the scientific literature regarding its mode of action. Nor was there a specific definition of the meaning of the term or description of methods of measuring shortening power. Not until 1921 did Davis (11) design an apparatus to measure shortening power and

hence define shortening in terms of measurable units. As Davis expressed it, "That material has the greatest shortening power which, when baked in a dough under standard conditions, gives to the product a minimum breaking strength and a minimum crushing strength (11, page 799)."

Today the relative shortening power of fats can be expressed with considerable accuracy in figures obtained by the use of Davis's shortometer (11) as originally constructed or as modified by other workers, especially Bailey (31). In the use of these devices, the amount of force necessary to break or crush a cookie or a sample of pastry is observed. The most commonly used shortometer today is Bailey's (31). It consists of a spring scale. Mounted parallel on the pan are two inverted U-shaped bars. The test sample is placed on these bars and a third bar parallel to the other two and centered between them is drawn down slowly by means of a motor. The increasing force is applied until the sample breaks. The force in grams as recorded on the scale is the breaking strength.

While it has been difficult to measure and evaluate the relative shortening power of fats, it is much more difficult to arrive at an adequate explanation of the mechanism of their behavior. The action of shortening may be described in a general way by saying that in flour mixtures the fat will separate and lubricate the flour particles and so prevent the formation of long, continuous strands of gluten which have great cohesive power.

In 1923, Platt and Fleming (30) attempted to relate physical characteristics of fat to shortening power. They indicated that "the action of shortening is 'physical' rather than 'chemical' (30, page 392)." This may be illustrated by the studies in which vaseline and mineral oil had a shortening power similar to that of butter and margarines. The factors that Platt and Fleming studied in relation to shortening power included viscosity, surface tension, melting point, plasticity and unsaturation.

Platt and Fleming stated that viscosity and surface tension are not of prime importance. Petroleum oils with viscosities similar to those of true fats have different shortening powers. Oils having only slightly different surface tensions differ greatly in shortening power.

Melting point values cannot be considered alone, as illustrated by the use of coconut oils that had the same melting point as butter or lard but were less satisfactory as shortening agents. Melting point and plasticity, both of which are considered factors influencing shortening power, are related. Fats with a high melting point cannot be spread easily throughout the flour mixture and have little shortening power. Melting point and plasticity are influenced by the percentage of unsaturated glycerides.

The property of plasticity may be absent, as in oil. In such a case a relatively high shortening power may be due to a high proportion of unsaturated compounds.

Iodine values cannot be used as an indication of shortening power. Iodine values do reflect the degree of total unsaturation, and this in

turn is related to plasticity. A plastic fat is capable of covering flour particles, thus inhibiting gluten formation or development. In a given glyceride the fatty acid chains can cover the same area whether each chain contains one double bond or more than one (30). A fat containing large amounts of linoleic acid has a higher iodine value than a fat composed mainly of oleic acid and yet their shortening power may be similar.

Fisher (32) used the Bailey shortometer to assay shortening power and related this to the physical and chemical constants congealing point, titer and iodine value. Fisher concluded that the congealing point decreased as the relative shortening value increased. The relationship, however, does not appear to be consistent once the fats are hydrogenated or compounded with hydrogenated fats. Iodine number and titer (a value similar to congealing point but determined on the fatty acids) did not show any consistent relationship with relative shortening value. Cawood (nee Fisher) in further work (33) found that as the percentage of fat in wafers increased, the breaking strength decreased. As the level of each of the shortenings used in the wafers was changed, the changes in breaking strength did not necessarily give the same relative shortening values. The relative shortening value of a fat used in wafers at one level cannot be used to calculate the relative shortening value of that fat used at a different level. Matthews and Dawson (34) reported similar findings.

Lowe et al. (10) reported an extensive study of various factors influencing shortening power. Of the physical and chemical characteristics of fats, these workers considered covering power to be the principal factor relating to shortening power. They listed five factors that influenced covering power: concentration, kind and temperature of the fat, manipulation and other ingredients and their concentration.

Lowe et al. found that, with the fats used, breaking strength was related inversely to the iodine number but the degree of unsaturation was not the sole determinant of shortening power. Breaking strength and refractive index also showed an inverse relationship, whereas the congealing point and melting point were directly related to breaking strength. The iodine number for some fats may be calculated from the refractive index. It appears that the presence of double bonds enables the fat to cover more surface per molecule and causes the fat to adhere more closely to the surface so that more force is required for two layers of the dough to come in contact with each other through the shortening (30). Fatty acids with two double bonds, however, do not cover more area per molecule than those with one double bond (30).

Harvey (35) evaluated the effect of changes in plasticity of fats on relative breaking strengths of pastry wafers. The changes made included increasing the temperature of use of the shortening, adding oil to the shortening, decreasing the degree of hydrogenation and mechanically working the shortening before use. Increasing the mixing

temperature decreased the relative breaking strength. Substituting increased amounts of oil for the hydrogenated oil decreased the relative breaking strength. Increasing the plasticity through a decrease in the extent of hydrogenation of an oil, as well as through mechanical mixing of the fat, decreased the relative breaking strength.

Hornstein and co-workers (3) believed that plasticity is a factor related to the shortening power of fats. They found a highly significant correlation between breaking strength and the consistency of the worked fat. Breaking strength did not correlate with iodine value, free fatty acids, melting point or congealing point of fat. Hornstein et al. suggested that melting points of the component glycerides are a major factor in the consistency of a shortening. In a given series of fats of similar fatty acid composition the ratio of liquid to solid glycerides would be related to iodine value. After hydrogenation this relationship would no longer be valid due to several factors including the occurrence of elaidinization. Elaidinization methods have been patented for production of plastic shortening from oils without loss of unsaturation (15, 28). This change in consistency is caused by the presence of trans isomers, which have a higher melting point but still react with iodine. Hornstein and co-workers stated that, on the basis of existing evidence, the theory that unsaturation plays a deciding role in determining shortening power is no longer valid.

Data obtained by the Institute of American Meat Packers were published in 1934 in the handbook "Lard" (36). The revised edition,

containing the above and additional information obtained in the 1940s, did not reach publication. The only recent article comparing current commercial fats and oils was authored by Matthews and Dawson (34). These workers found specific gravity and viscosity of oils to be positively related to shortening power in wafers containing 41 percent lipid.

Evaluation of shortening power. Plain pastry is a satisfactory product in which to test shortening power. This product, which has a high percentage of fat, contains relatively few other ingredients: salt, water and flour. Mixing is a fairly simple technique that does not involve creaming or emulsifying ability of the fat. The preparation is adapted to machine mixing.

There are still many variables to control. Swartz (37) reviewed several of the factors affecting the breaking strength of wafers. The most extensive study of such factors was reported by Lowe and co-workers (10), who studied temperature of ingredients, length of cooling time, time and temperature of baking, and rate and extent of mixing. Other factors that are of interest are kind of flour (38), room temperature and humidity (38), method of rolling (39), size of test wafers (37) and material of baking sheet (40). Because of this large number of possible variables, absolute values are difficult to compare from one laboratory to another or from one time to another. It is possible to obtain the same relative results. Fisher (32) first used the term "relative

shortening value." Harvey (35) believed that the correct term should be "relative breaking strength." Relative breaking strength of a sample is expressed as percentage of the average breaking strength of a control set of wafers.

Summary. Shortening power has been studied in relation to a variety of physical and chemical properties of the fat. Among these properties are congealing point, plasticity, ratio of solid to liquid glycerides, degree of unsaturation and iodine value. No single characteristic was identified as the sole determinant of shortening power of fats. One factor which was not reported as having been studied in relation to shortening power is elaidinization. Elaidinization can occur during hydrogenation, resulting in the formation of more stable, higher melting isomers.

III. PROCEDURE

Preparation of Lipid Samples

Procurement of lipids. Oleic acid and triolein, of USP and Technical grade respectively, were obtained from a chemical supply house. The commercial cottonseed oil was purchased from a local food market in bulk. Upon receipt, the lipids were stored at -20C.

Elaidinization. The elaidinization method of Litchfield and co-workers (19) was used for preparation of the elaidinized lipids. A 350 g sample of oleic acid or of triolein was placed in a 2 liter reaction vessel which had been flushed with nitrogen. The reaction vessel was purged again with nitrogen. The sample was heated to 65C with constant stirring. Seventeen milliliters of 6 M nitric acid solution and 25 ml of 2 M sodium nitrite solution were added through a dropping funnel. The sample was heated for 30 min in a constant temperature waterbath. At the end of the heating period, the vessel was removed from the waterbath, while being continously flushed with nitrogen; 400 ml of petroleum ether (boiling range 30-60C) were added. Two hundred milliliters of water were added, and the sample was transferred to a separatory funnel for removal of the aqueous layer. Washing with water was repeated three times and the petroleum ether extract was dried 1 hr on sodium sulfate. The dried sample was transferred to a centrifuge bottle containing 100 g of silicic acid that

had been heated for 4 hr at 110C. The slurry was mixed 10 min with a magnetic stirrer, then centrifuged for 10 min at 1000 x G. The supernatant was decanted into a 1000 ml boiling flask, which then was flushed with nitrogen and refrigerated. The silicic acid in the centrifuge bottle was resuspended in 50 ml petroleum ether and mixed and centrifuged as described above. The supernatant was added to the first extract. Washing of the silicic acid was repeated three times. Three samples of oleic acid and three of triolein were subjected to the elaidinization procedure, and the three extracts of each lipid were pooled. Solvent was removed from each sample on a rotary evaporator. Each lipid sample was transferred to a brown bottle, flushed with nitrogen and stored at -20C until used.

Sample preparation. The lipid samples to be tested were commercial vegetable oil, oleic acid, triolein, and mixtures of oleic acid plus elaidinized oleic acid and of triolein plus elaidinized triolein (Table I). Samples were mixed thoroughly. Ten 16.4 g portions were

TABLE I

Composition of Lipid Samples

Component	Sample 1	2	3	4	5	6	7	8	9
	%	%	%	%	%	%	%	%	%
Cottonseed Oil	100								
Oleic Acid		100	90	70	0				
Elaidinized Oleic Acid		0	10	30	100				
Triolein						100	90	70	0
Elaidinized Triolein						0	10	30	100

removed from each sample and stored under nitrogen at -20C for use later in wafer preparation.

Composition Analyses

Methylation. A portion of each lipid sample was converted to fatty acid methyl esters. Approximately 0.5 ml of each sample was placed in a 15 ml conical centrifuge tube. Five milliliters of 1.1 N hydrochloric acid-methanol were added. The samples were refluxed for 1.5 hr in a waterbath held at approximately 72C. During refluxing the samples were mixed at least three times. After cooling 2 ml of water and 6 ml of petroleum ether (boiling range 30-60C) were added to each tube. This mixture was transferred to a 30 ml separatory funnel. The funnel and contents were shaken for 1 min. The lower aqueous phase was removed. Washing with 2 ml of water was repeated three more times. After the addition of 0.5 g sodium sulfate and 0.5 g silicic acid, the sample was allowed to stand for 1 hr, after which it was drained into a centrifuge tube, flushed with nitrogen, tightly stoppered and stored at -20C. All solvents used in each procedure described had been dried and redistilled.

Gas-liquid chromatography. The fatty acid composition of the nine methylated samples was determined by gas-liquid chromatography (GLC). A model 61C Barber-Colman gas chromatograph with argon ionization detector (radium) was used. An aluminum column, 7 ft x 4 mm, was

packed with 13 percent diethylene glycol succinate polyester on 80-100 mesh Gas-Chrom P*. Operating conditions were as follows: column temperature, 186C; split temperature, 227C; cell temperature 222C; flash heater temperature, 232C; cell voltage, 900; gas pressure, 17 psi; attentuation, 16; and sensitivity, 10. Chromatographic standards were used for identification.

Peak "areas" representing fatty acids present were calculated by multiplication of the height of each peak by the width at half-height. The percentages of specific fatty acids were calculated from the "areas" of the peaks present.

Thin-layer chromatography. GLC conditions did not separate the cis-trans isomers of methyl esters having equal chain length. The proportions of trans monoenes were determined by argentation thin-layer chromatography (TLC) based on methods of Morris (25, 26, 41) and of Barrett and co-workers (42). A slurry of 30 g of Silica Gel G in 60 ml of a 12.5 percent silver nitrate solution was used for coating thin-layer plates (20 x 20 cm) with a 0.3 mm layer. The coated plates were air dried for 10 min and oven dried for 60 min at 110C under a nitrogen atmosphere. All plates were stored in a covered metal container over a desiccant until used. Lanes 2.2 cm wide were drawn in preparation for spotting (43). On the vertical lane lines, small crossbars (2 mm) were

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*Applied Science Laboratories, Inc.

drawn 2.5 cm from the lower horizontal edge. Each lane was spotted with 10-30 µl (5-10 µg) methyl ester solution 2.5 cm from the bottom edge. Spotting was carried out under a stream of nitrogen. The plates were developed to 2.2 cm from the top edge in a chamber that had been saturated for 51 min with a piece of Whatman No. 3 filter paper (22 x 23 cm) suspended in the developing solution (petroleum ether, diethyl ether and acetic acid, 90/10/0.25, v/v/v). The time required for development varied with room temperature, averaging about 30 min. After development the plates were air dried for 10 min then sprayed with 50 percent sulfuric acid solution and charred for 15 min at 180C (44, 45). After the plates had cooled a chromatogram was made of each lane on a Photovolt Densitometer 425 with a strip chart recorder. The percentages of trans fatty acids were calculated from the areas under the appropriate peaks. Chromatographic standards were used for identification.

Physical Measurements of Lipid Samples

Viscosity. Viscosity measurements of the nine lipid samples were made in triplicate with a Brookfield Synchro Lectric Viscometer, Model LVF. Samples of the lipid material were brought to room temperature or higher until they could be poured into 250 ml electrolyte beakers. Viscosity measurements were made at 42C. A waterbath was used to maintain the temperature. Readings were taken with the no. 1 spindle at 30 and 60 rpm. The values for each sample were averaged.

Surface and interfacial tension. Surface tension and interfacial tension were determined in triplicate with a Cenco-DuNoüy interfacial tensiometer model no. 70545. Measurements were made at 42C. For each determination 25 ml of lipid sample, 25 ml of distilled water and a crystallizing dish, 56 cm in diameter, were brought to constant temperature in a waterbath. In the measurement of interfacial tension 25 ml of distilled water were poured into a crystallizing dish. The ring was lowered about 5 mm into the water. Twenty-five milliliters of the lipid sample were poured carefully onto the water. The height of the ring was adjusted until it was in the interface. The torsion of the wire attached to the lever arm holding the ring was increased while the dish was lowered thus keeping the lever arm in a neutral position. When the film at the interface broke, the interfacial tension in dynes per cm was read from the dial. Surface tension values were determined on the lipid samples in a similar manner.

Melting point. The melting points of the lipid samples were determined by the AOCS method Cc1-25 (46), except that the filled capillary tubes were held at -20C instead of 4C before being tested.

pH values. The pH of the ground wafers made with each of the nine lipids was determined for three replications. A 10 g sample of each of the wafer types was slurried with 25 ml of distilled water.

Shortening Power Determinations

The pastry formula (Table II) was patterned after that used by

TABLE II

Pastry Formula

Component	Amount	Percent by Weight of Flour
Flour, Soft Wheat, All Purpose	41.0 g	-
Lipid	16.4 g	40
Salt	1.0 g	2
Water, Distilled	10.0 ml	25

previous workers (10, 32, 37, 38, 39). The lipid was weighed in advance and stored under nitrogen at -20C until the morning of its use. The flour and salt were weighed and stored in sealed containers at room temperature. The mixing bowl, beater, water, salt, flour and lipid material for each batch of wafers were placed in a constant temperature waterbath at 42C 15 min prior to mixing. A table of random numbers was used to determine the order of preparation of the nine batches of pastry in each of 10 replications.

The wafers were prepared in an air-conditioned laboratory. Temperature and relative humidity were recorded at half-hourly intervals. (Table VII, Appendix).

All mixing was done in a Kitchen Aid Mixer, Model 3-C, at speed 2. The flour and salt were blended for 20 sec. The lipid was added during a period of 6 sec. The lipid, flour and salt were mixed 35 sec. The water was added all at once and the mixing continued for 60 sec;

during the first 10 sec the cylinder was drained. The dough, which had formed a ball during mixing, was removed and rolled between waxed paper strips placed in a frame of two metal cleats 2.3 mm thick, parallel and 7.6 cm apart. A template with parallel slots 3.8 cm apart was used as a guide in cutting the dough strip into 10 wafers. A scorer, 2.5 x 3.3 cm, with 48 stainless steel blades was used for perforating the wafers. The perforated wafers were transferred to an aluminum baking sheet by inversion of the lower strip of waxed paper. Slight finger pressure on alternate wafers caused five of them to stick to the sheet. The waxed paper was raised and the other five wafers adhering to it made a second row. The wafers again were perforated, then baked for 4.5 min at 218C in a rotary hearth oven. The baked wafers on the baking sheet were placed on a wire rack. After two hours of cooling, the breaking strength was determined on a Bailey Shortometer. Relative breaking strength values were calculated (35).

Moisture Determinations

Moisture determinations were made on all dough and wafer samples. The dough left after rolling was cut into approximately 3 mm squares. After breaking strength measurements, the wafers were crushed between pieces of waxed paper and mixed. Approximately 2 g samples were weighed into previously dried and weighed moisture pans. Duplicate determinations were made on each sample. The samples were air dried overnight and oven dried for 4 hr at 110C. After cooling the pans were reweighed. Moisture percentages were calculated from weight losses (47).

Statistical Analyses

All statistical analyses were carried out at the computer center. Data from all variables were submitted to simple data analyses (averages, standard deviations and ranges), simple correlations, multiple regression and analysis of variance (48). In addition, Duncan's multiple range test was used for comparing the test sample averages, whereas the Dunnett's test was used for comparing all of the averages with the control average (48).

IV. RESULTS AND DISCUSSION

Lipids

The fatty acid composition of the methylated samples is presented in Table III. The main component of the control sample, cottonseed oil, was linoleic acid (75.8 percent). Samples other than the control were composed mainly of C18:1. Measurable peaks of C18:2 were obtained for only three samples; therefore, C18:2 values were not included in the statistical analyses. Analysis of variance showed the concentrations of C14:1 and C16:1 to be higher ($P < 0.05$ and 0.01, respectively) in the oleic acid than in triolein and that of C18:1 to be higher ($P < 0.01$) in the triolein. According to the results of argentation TLC, the elaidinization process resulted in 60.6 percent trans monoenes in the oleic acid as compared with 42.7 percent in the triolein.

The concentration of total monoenes remained essentially unchanged, whereas the level of trans isomers increased as expected as increasing amounts of elaidinized samples were substituted for the oleic acid and triolein. The level of trans isomers was greater ($P < 0.01$) for the completely substituted oleic acid and triolein than for other levels of substitution.

Physical Measurements

Melting point, surface tension, viscosity and interfacial tension of the lipids and pH of the wafers are reported in Table IV. Melting

TABLE III

Fatty Acid Composition of Lipid Samples Used in Wafers

Sample	Substitution Level of Elaidinized Lipid %	Fatty Acids[a] C14:0 %	C14:1 %	C16:0 %	C16:1 %	C18:1 %	C18:2 %	Total Monoenes %	Trans[b] Monoenes %
Control	0	0.2	0.0	13.2	0.6	10.2	75.8	10.8	0.0
Oleic Acid	0	1.8	2.4	1.4	7.4	86.9	Trace	96.6	7.3
Triolein	0	1.3	0.4	1.4	3.5	93.4	Trace	97.4	3.2
Oleic Acid	10	1.7	2.3	0.6	10.0	84.8	0.5	97.2	12.1
Triolein	10	1.2	0.6	0.7	2.8	93.9	0.9	97.2	5.5
Oleic Acid	30	2.0	2.5	1.4	9.4	84.6	Trace	96.6	20.0
Triolein	30	2.1	1.3	1.3	3.4	91.8	-	96.6	11.6
Oleic Acid	100	3.4	3.4	0.5	9.0	83.0	-	95.6	60.6
Triolein	100	2.6	0.6	1.5	5.0	90.0	Trace	95.6	42.7

[a]Number of carbons;number of double bonds.

[b]Percent of total monoenes

TABLE IV

Melting Point, Surface Tension, Viscosity and Interfacial Tension of Nine Lipid Samples and pH of Wafer Slurries

Sample	Substitution Level of Elaidinized Lipid %	Melting Point C	Surface Tension at 42C dynes/cm	Viscosity at 42C cp	Interfacial Tension at 42C dynes/cm	pH
Control	0	2.7	34.6	29.0	21.3	4.9
Oleic Acid	0	8.6	32.4	15.6	14.2	4.7
Triolein	0	5.5	33.7	33.9	7.5	4.9
Oleic Acid	10	11.6	33.3	16.7	13.9	4.7
Triolein	10	6.6	34.0	33.6	7.9	4.9
Oleic Acid	30	18.4	33.3	15.8	14.2	4.7
Triolein	30	20.6	34.8	34.5	9.6	4.9
Oleic Acid	100	31.7	33.3	14.4	14.4	4.7
Triolein	100	26.6	34.2	32.0	21.7	4.8

point increased with increasing level of substitution, the averages being higher ($P < 0.05$) at the 30 and 100 percent levels of substitution than at the 0 and 10 percent levels.

The surface tension and viscosity of the fatty acid samples were lower ($P < 0.01$) than those of the triglyceride samples. Values for interfacial tension did not differ consistently, but the triglyceride values were lower than those of the fatty acids except at the 100 percent level. The pH of the wafers containing fatty acids was lower ($P < 0.01$) than that of the wafers containing triglycerides.

Simple correlations were made of all data from physical and lipid compositional measurements. The significant correlation coefficients are reported in Table V. Melting point correlated positively with the percent of trans isomers. Surface tension showed positive correlations with pH of the wafers and viscosity of the lipids and negative correlations with the percent C14:1 and C16:1. Viscosity measurements also correlated negatively with the percent C14:1 and C16:1 and positively with pH of the wafers. The pH of the wafers correlated negatively with the percent C14:1 and C16:1.

Breaking Strength

The averages and standard deviations of breaking strength values of wafers made from nine lipid samples are reported in Table VI. The Dunnett's test was used for comparing all of the averages with the control average. These results are not shown; only the breaking

TABLE V

Correlation Coefficients of the Related Measurements[a]

	r Values									
						Percent				
Variable	Breaking Strength	Melting Point	pH	Viscosity	Trans Isomers	C14:0	C14:1	C16:0	C16:1	C18:1
Melting Point	0.74				0.92	0.93				
Surface Tension			0.80	0.80			-0.69		-0.74	
Viscosity			0.94				-0.88		-0.86	
pH							-0.88		-0.94	
Percent										
Trans	0.80					0.89				
C14:0							0.69	-0.68		
C14:1									0.90	
C16:0										-0.98
Percent Moisture										
Dough		0.86	-0.72		0.87	0.96	0.73		0.72	
Wafer								-0.92		0.94

[a] r-values of 0.80 or greater are significant at the $P \leq 0.01$ level; other values are significant at the $P \leq 0.05$ level.

TABLE VI

Averages and Standard Deviations of Breaking Strength of Wafers and of Percent Moisture in Doughs and Wafers Made from Nine Lipid Samples

Sample	Substitution Level of Elaidinized Lipid %	Breaking Strength g ± sd	Percent Dough Moisture % ± sd	Percent Wafer Moisture % ± sd
Control	0	59 ± 9.5	18.3 ± 0.29	3.7 ± 0.35
Oleic Acid	0	49 ± 5.1	19.0 ± 0.43	4.8 ± 0.23
Triolein	0	62 ± 11.8	18.6 ± 0.39	4.7 ± 0.48
Oleic Acid	10	46 ± 4.4	18.9 ± 0.39	4.8 ± 0.36
Triolein	10	59 ± 5.0	18.6 ± 1.03	4.9 ± 0.54
Oleic Acid	30	52 ± 6.2	18.9 ± 0.24	4.9 ± 0.36
Triolein	30	68 ± 9.2	18.8 ± 0.27	4.9 ± 0.38
Oleic Acid	100	89 ± 24.1	19.4 ± 0.16	4.6 ± 0.59
Triolein	100	79 ± 9.7	19.2 ± 0.30	5.1 ± 0.35

strength values of the wafers containing elaidinized lipid substituted at the 100 percent level were higher ($P < 0.01$) than those of the control.

An analysis of variance for a randomized complete block design was used for testing differences among the breaking strength averages of the oleic acid and triolein samples. Except at the 100 percent level of substitution the use of triolein in preparation of the wafers resulted in a greater ($P < 0.01$) breaking strength than did the use of oleic acid. The level of substitution of elaidinized sample affected the breaking strength with only the 100 percent level of substitution resulting in breaking strengths that were greater ($P < 0.01$) than those at other levels of substitution.

In the analysis of variance of breaking strength, an interaction between type of lipid and level of substitution was found ($P < 0.01$); the effect of level of substitution was greater with fatty acid than with triglyceride. This may reflect the fact that a given level of substitution of elaidinized lipid did not give the same percent of trans isomers in oleic acid and triolein samples.

Relative breaking strength values (Table VIII, Appendix) also were used in statistical analyses. The results did not differ from those obtained with the use of breaking strength values.

Simple correlations of breaking strength and all other variables measured showed breaking strength to correlate ($P < 0.01$) with the percent trans isomer content of the lipid and with the melting point of the lipid (Table V, page 27).

Moisture

The averages and standard deviations of percent moisture in doughs and wafers are reported in Table VI, page 28. The Dunnett's test was used for comparing the test sample averages with the control sample average. The moisture levels of the oleic acid sample doughs and wafers were higher ($P < 0.05$) than those of the control doughs and wafers. Of the triolein sample doughs, only the dough with elaidinized lipid substituted at the 100 percent level was higher ($P < 0.01$) in moisture content than the control dough. Moisture levels of the triolein sample wafers were greater ($P < 0.01$) than those of the control wafers.

An analysis of variance for a randomized complete block design was used to test for differences in moisture content of doughs and wafers for samples excluding the control. The percent moisture of the doughs made from fatty acids was higher than that of doughs made from triglycerides. Significant differences were not detected among the levels of moisture of the wafers containing oleic acid and triolein samples. Duncan's multiple range test indicated that the percent moisture of doughs made from elaidinized oleic acid and triolein at the 100 percent level of substitution was higher ($P < 0.01$) than that of the other samples.

The percent moisture of the doughs was correlated ($P < 0.01$) with melting point of the lipid samples and pH of the wafers (Table V, page 27). No significant correlation coefficients were found for the percent moisture of the wafers and physical measurements. The percent

moisture of the doughs and wafers showed an interesting relationship to the composition of the lipids. The dough moisture was correlated with the percent of trans isomers and C14:0 ($P < 0.01$) and of C14:1 and C16:1 ($P < 0.05$) in the lipid. The percent moisture of the wafers was correlated ($P < 0.01$) with the remaining major components of the lipids, C16:0 and C18:1.

Relationships Among Measurements

Relationships among various measurements made on the lipid samples, doughs and wafers may reflect the type of lipid or the level of substitution of elaidinized material. Viscosity and surface tension of the lipids, pH of the wafers and percent moisture of the doughs containing the lipids appear to be related to the type of sample, fatty acid or triglyceride; melting point of the lipid and, again, percent moisture of the doughs appear to be related to the level of substitution of elaidinized material. It, therefore, would seem appropriate to discuss these various relationships.

The viscosity and surface tension of the fatty acids and the pH of the wafers containing fatty acids were lower than the same properties in the triglycerides and wafers containing them. The moisture levels of the doughs containing fatty acids were higher than those of the doughs containing triglycerides. Viscosity and surface tension of the lipid samples and the pH of wafers made from the lipid samples may affect the breaking strength of wafers through a relationship with gluten hydration. The pH of a mixture does affect the

hydration of gluten. In this study, the degree of hydration as indicated by the percent moisture in the dough increased with decreased pH.

The effect of the carboxyl group of the fatty acid on the breaking strength may be evaluated. Lowe and co-workers (10) substituted various amounts of oleic acid for lards in the preparation of wafers. The breaking strength of wafers decreased with increasing amounts of fatty acid. They described the effect of adding oleic acid as being related to increased adsorbability and increased coating ability due to double bonds or to the shortening effect of fatty acids acting upon the gluten. As carboxyl groups and double bonds were increased simultaneously, they were not able to specify which factor was responsible for the effect. In the present study, wafers containing the fatty acid did have lower breaking strengths than the corresponding wafers containing triglyceride except at the 100 percent level of substitution. It would appear that the presence of the carboxyl group also may have a shortening effect. Sullivan et al. (49) reported that the inclusion of oleic acid in wafers produced short strands of tough, brittle gluten.

Higher fluidity and greater spreading power of the fatty acids are reflected in their having lower viscosity and lower surface tension than the triglycerides. This greater fluidity may be related to the lower breaking strength of the wafers containing fatty acids.

Melting point of the samples and percent moisture of the doughs were related to the level of substitution. The melting points of

samples substituted at the 30 and 100 percent levels were higher ($P < 0.05$) than those of the other samples and different from each other. Melting point can be related to the degree of unsaturation and hence iodine value. Lowe et al. (10) reported that iodine value was related to breaking strength. In a given series of similar fats this may hold true. Hornstein et al. (3) indicated that after hydrogenation the relationship of breaking strength and iodine value is no longer proportional. They suggested that this change may be attributable to the occurrence of trans isomers. In the present study melting point reflected the presence of trans isomers. As the melting point increased or as the percent of trans isomers increased the breaking strength of the wafers increased, indicating that geometric configuration must be related to shortening power. This finding supports the suggestion of Hornstein et al. (3) that the iodine value of lipids that are hydrogenated cannot be used to predict shortening power adequately.

The percent moisture of the dough containing lipid substituted at the 100 percent level was higher ($P < 0.01$) than that of the dough containing lipid substituted at lower levels. This corresponds to the significant differences detected in breaking strength. In general the breaking strength of wafers tended to increase with an increase in the moisture level of the dough.

It may be possible to predict the shortening power of a fat from various physical measurements. In this study percent of trans isomers and pH of the wafers accounted for most of the variation in breaking

strength. In the multiple regression analysis, the correlation coefficient was 0.799 with the percent trans isomers, increased to 0.979 with pH of the wafers, to 0.984 with surface tension, to 0.989 with percent moisture of the wafers, to 0.998 with percent moisture of the dough and to 1.000 with melting point of the lipid. The regression equation for breaking strength (y) was: y = -742.20 + 0.466 percent trans isomers + 148.27 pH of wafers - 6.194 surface tension - 10.788 percent moisture of wafers + 17.890 percent moisture of the dough + 0.495 melting point.

Further work in this area should be conducted to determine the effect of trans isomers on wafers prepared at room temperature. However, it does appear that the presence of trans isomers influences the shortening power of lipids.

V. SUMMARY

The effect of cis-trans isomers on the shortening power of lipids was investigated. Lipid samples studied included: a commercial cottonseed oil (control), oleic acid, triolein, elaidinized oleic acid, elaidinized triolein, two samples in which elaidinized oleic acid was substituted at different levels for oleic acid and two samples in which elaidinized triolein was substituted at different levels for triolein. Breaking strength of plain pastry wafers was used for estimating the shortening power. Melting point, surface tension, interfacial tension and viscosity measurements were made on the lipid samples. Lipid composition analyses included gas-liquid chromatographic determination of fatty acids and thin-layer chromatographic determination of percent trans isomers.

Wafers containing fatty acids had lower breaking strengths than those containing triglycerides. Breaking strengths of wafers containing samples substituted at the 100 percent level of elaidinized material were greater than those of wafers containing samples substituted at lower levels. Melting points of the lipids were positively correlated with breaking strength and with percent trans isomers. Surface tension and viscosity of fatty acid samples were lower than those of triglyceride samples. Interfacial tension showed no significant correlation with any other measurement. The moisture levels of the doughs made from fatty acids were higher than those made

from triglycerides. Doughs containing elaidinized lipids at the 100 percent level of substitution were higher in moisture content than the other doughs.

Relationships among various measurements made on the lipid samples, doughs and wafers may reflect the type of lipid or the level of substitution of elaidinized material. Viscosity and surface tension of the lipids, pH of the wafers and percent moisture of the doughs containing the lipids appear to be related to the type of sample, fatty acid or triglyceride, whereas melting point of the lipid and percent moisture of the doughs appear to be related to the level of substitution of elaidinized material. Viscosity and surface tension of the lipid samples and the pH of wafers made from the lipid samples may affect the breaking strength of wafers through a relationship with gluten hydration. It would appear that the presence of the carboxyl group of the fatty acid in wafers may account for their having lower breaking strength than the wafers containing the same fatty acid as a triglyceride. Melting point of the lipids, which was related to the level of substitution, reflected the presence of trans isomers. Breaking strength of wafers increased as melting point and concentration of trans isomers in the lipids increased. In general the breaking strength of wafers tended to increase also with an increase in the moisture level of the dough. A multiple regression equation is presented for predicting breaking strength values.

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APPENDIX

TABLE VII

Averages and Standard Deviations of Daily Temperature and Relative Humidity for Each Replication

Replication	Daily Average Temperature °C	Daily Average Relative Humidity %
1	24 ± 1.3	56 ± 2.0
2	24 ± 1.2	54 ± 2.7
3	24 ± 1.3	54 ± 2.2
4	24 ± 1.2	57 ± 2.1
5	24 ± 0.8	57 ± 2.0
6	24 ± 1.3	57 ± 1.8
7	23 ± 1.4	60 ± 2.5
8	24 ± 1.8	51 ± 2.8
9	24 ± 1.4	51 ± 1.9
10	24 ± 0.4	48 ± 1.5
Overall Average	24 ± 0.3	54 ± 3.7

TABLE VIII

Averages and Standard Deviations of Relative Breaking Strength Values of Wafers Made from Nine Lipid Samples

Sample	Substitution Level of Elaidinized Lipid	Breaking Strength ± Standard Deviation
	%	%
Control	0	100 ± 0.0
Oleic Acid	0	86 ± 15.0
Triolein	0	108 ± 26.6
Oleic Acid	10	80 ± 16.6
Triolein	10	103 ± 18.0
Oleic Acid	30	90 ± 16.6
Triolein	30	117 ± 19.8
Oleic Acid	100	153 ± 41.6
Triolein	100	137 ± 22.9

VITA

The author is Aneta Joyce Ostrander, the wife of K. H. Ostrander. She was born in Wallowa, Oregon. She received the B. S. degree from Oregon State University and the M. S. degree from Purdue University. She was initiated into Sigma Delta Epsilon and Sigma Xi at Purdue University and promoted to full membership in Sigma Xi at the University of Tennessee. She was selected as an Outstanding Young Woman of America. Lipid research is her field of interest. She has published articles in Poultry Science, Journal of American Oil Chemists' Society and American Meat Institute Foundation Bulletins and Circulars. She has worked at the American Meat Institute Foundation in Chicago, and at Midwest Research Institute in Kansas City, Missouri, and taught at the University of Kansas in Lawrence.